

Effects of Varying Mixtures of Dietary Stearic and Linoleic Acids
on Fatty Acids in Plasma Membranes of Transplanted Tumors

An Honors Thesis (ID 499)

by

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ABSTRACT

The fatty acid composition of plasma membrane fractions of mammary adenocarcinomas from Strong A female mice fed diets varying in the amounts of linoleic and stearic acid were studied. Studies have indicated that linoleic acid is a tumor promoter and stearic acid is a tumor inhibitor. Studies have also shown that the plasma membrane plays an important role in the cell's responses to growth factors and in the development of the tumor.

The effects of varying amounts of dietary 18:0 and 18:2 on the plasma membrane were studied. The purpose of the project was to determine the effects of these dietary fatty acids on the concentration of fatty acids in the plasma membrane.

The Strong A mice were fed a high fat (15%) or low fat (5%) diet. The high fat diets were: SA-1 (13.2% 18:0, 0.8% 18:2), SA-4 (10.3% 18:0, 3.0% 18:2), and SAF (0.4% 18:0, 11.4% 18:2). The low fat diets were: STOCK (0.3% 18:0, 1.5% 18:2) and CO (0.1% 18:0, 3.0% 18:2).

A decrease in linoleic acid content was noted in all membranes except for the large tumors from mice fed the SAF diet which contained 3.5% as compared to 2.7% linoleic acid in stock tumors. There was also a very large increase in the amount of 18:0 in the SA-4 large (24.1%) and small (16.9%) as compared to 8.1% for the stock tumors. In the rest of the dietary groups there was not a significant difference in the amount of 18:0 as compared to the stock, although there was a small increase in each diet.

INTRODUCTION

It is known that fats are promoters of mammary tumors. It is also known that plasma membranes play an important role in the cell's responses to growth factors and in the development of the tumor. Recently the membrane has been considered a serious location for antineoplastic therapy. By altering the structure of the membrane, Burns and Spector were able to change tumors' responses to various environments which implies that membrane therapy is possible.

Many studies have examined the effects of various polyunsaturated fats on the incidence and growth of tumors. It has been discovered that there is an increase in proliferation of cancer with an increase in polyunsaturated fat in the diet. It was also discovered that diets rich in saturated fatty acids caused a decrease in the proliferation of cancer. Specifically, linoleic acid, which is a polyunsaturated fatty acid, has been shown to be a tumor promoter. Stearic acid, on the other hand, is a saturated fatty acid and has been shown to inhibit tumor production.

Although it is known that polyunsaturated fat causes an increase in tumor production, little work has been done on the effects of specific fatty acids on the plasma membrane. The effects of various amounts of linoleic acid and stearic acid in the diet have been studied, but not their effects on the plasma membrane. In this investigation the effects of varying amounts of linoleic acid and stearic acid, in isocaloric diets, on plasma membranes were studied.

LITERATURE REVIEW

History of the Cell Membrane

The membrane of a cell plays a much more important role than just compartmentalization of the cell. It controls the movement of substances into and out of the cell, plays an important role in detection of external signals and serves as the locus of specific functions (1). Charles Overton, in the 1890's, was aware that cells seemed to be enveloped by some sort of selectively permeable layer that allowed the passage of some substances but not others. Working with cells of plant root hairs, Overton found that lipid-soluble substances penetrated readily into cells. From this study Overton concluded that the solubility properties of the cell membrane resembled those of fatty oil (1).

Another important advance came in the early 1900's when Langmuir studied the behavior of amphipathic phospholipids by spreading them out as a thin layer on a water surface. By dissolving the lipids in benzene and then allowing the benzene to evaporate, Langmuir developed the first lipid monolayer (1). In 1925, Gorter and Grendel used Langmuir's monolayer technique along with lipids they extracted from red blood cells and determined that there were two monolayers or one lipid bilayer (1). Unto this day the lipid bilayer is considered the basic structure of all membranes and serves as a permeability to most water-soluble

substances. It is from the results of their research that new insights have been developed on how to stop the proliferation of cancerous cells.

Biochemical Aspects

Linoleic acid, a polyunsaturated fatty acid, has been identified as a promoter of tumorigenesis and stearic acid, a saturated fatty acid, has been shown to increase the latency period (2). Erickson et. al. (3) found that the number of antibody forming cells appear to be inversely related to the levels of linoleic acid. His study dealt with the effects of dietary fat on the antibody response to a T-dependent antigen. On the other hand, Das et. al. (4) reported that polyunsaturated fats inhibit normal and tumor cell growth in vitro. The report also mentions that polyunsaturated fatty acids induced growth inhibition and autotoxicity to tumor cells by enhancing free radical generation. Free radicals can initiate lipid peroxidation and are toxic to tumor cells. Corwin et. al. (5) also found that tumorigenicity of their murine cell line increased as the polyunsaturated fatty acid level decreased. Yet, another strain which was grown on lipid depleted media showed a decrease in tumorigenicity with any decrease in polyunsaturated fatty acid level.

Kamiyama et. al. (6) found that there may be a unique tumor surface marker with chemical and immunological properties in common with those isolated directly from human breast adenocarcinomas. The surface marker was discovered by isolation

of a tumor surface antigen which induced the formation of antibodies which recognized the surface marker by the immune adherence hemagglutination test (6). Pitelka et. al. (7) found that the mouse mammary tumor cells in primary culture virtually always are contact inhibited. Pitelka's group also showed that one of their transplanted tumor lines, which formed far fewer junctions than typical mammary carcinoma and does not show contact inhibition in culture, is not exceptionally invasive and has not metastasized.

Research done by Burns and Spector (8) has shown that fatty acid modification of tumor membranes may make them more susceptible or sensitive to clinically useful therapeutic methods. Fatty acid modification is possible due to the fact that tumors derive most of their fatty acids from the extracellular fluid when an adequate supply of lipid is available. The fatty acids of cultured cells are modified by changing the type and amount of lipid contained in the growth medium. The fatty acids in the extracellular fluid are utilized preferentially and *de novo* synthesis is suppressed (8). Studies done by Bertino (9) show that some drugs that slow or stop cancer cells from proliferating are doing so by effecting the cell membrane. Bertino also states that cell membranes reflect the tissue of origin and therefore, they may have specialized features that can be exploited therapeutically. Cooper (10) mentions that it is possible that changes in fluidity may lead to susceptibility of disease. These findings lead to the fact that the cell membrane may be a plausible target for antineoplastic therapy.

Dietary Aspects

In a study done by Thomas (11), it was estimated that 30 percent of cancers may be caused by dietary factors. Thomas also stated that breast cancer is known to be estrogen dependent and estrogen production is related to obesity which is related to excess dietary fat. A study done by Chan and Cohen (12) showed that mammary tumor cell proliferation is stimulated when the prolactin:estrogen ratio is high and is inhibited when the ratio is low. It was also shown that chronic high fat intake elevated serum prolactin levels, which elevates the prolactin:estrogen ratio, and thereby promotes mammary tumor cell growth. In a study done by Sakaguchi et. al. (13), rats were fed a 5% fat diet containing linoleate or stearate to measure affects of dietary fat on colon carcinogenesis. The rats were given azoxymethane as a carcinogen and it was discovered that the rats on the unsaturated diet had significantly more colon cancer.

Becker and Bruce (14) demonstrated that the dietary level of essential fatty acids influences the utilization and metabolism of linoleic acid. Their study showed that animals fed low essential fatty acids retained 50% of the linoleic acid ingested as compared to 10-15% linoleic acid retained in normal and high essential fatty acid rats. It was also shown that there was a large amount of stearic acid retained in the low essential fatty acid diet mice and normal essential fatty acid diet mice and a large amount of linoleic acid retained in the high essential fatty acid diet mice. Siquel and Maclure (15) found that there was a decrease in the

amount of linoleic acid in individuals with essential fatty acid deficiency. Their study also showed an increase in oleic acid which shows the synthesis from saturated fatty acid precursors. A study done by Field et. al. (16) found that subjects with higher intakes of saturated fatty acids exhibited increased levels of total saturated fatty acids and decreased polyunsaturated fatty acids in adipose tissue. Field et. al. also stated that adipose tissue linoleic acid content is related to the nature of fat consumed.

Plasma membranes of cells are affected by dietary intake. Carlson et. al. (17) showed that with increased linoleic acid in the diet there tended to be a higher percent of linoleic acid in the red blood cell membrane. In a study done by Alan and Alan (18), it was discovered that membranes of rats that were essential fatty acid deficient were less fluid. Also, when these EFA deficient mice were fed a control diet for six weeks the fatty acid composition of membrane lipids was restored to normal values. This study illustrated the fact that diet does have an effect on plasma membranes. Burns and Spector (8) also illustrated this fact. In their study, they found that when a diet rich in saturated fat was fed to mice there was an increase in oleic acid (18:1) and a decrease in linoleic acid (18:2) within the membrane. Burns and Spector also found that there was a decrease in carrier-mediated activity in the mice fed a polyunsaturated fat.

Burns and Spector's (8) study indicated that lipid modification may have an effect on membrane function. This is very important due to the fact that membrane fatty acid

modifications may be achieved, either by growth of a tumor in animals fed different fat supplemented diets or in a culture by varying the lipids added to the medium. This ability to modify membranes may be a positive influence in the halting of proliferation of cancerous cells.

PROCEDURES AND METHODS

Materials

KHPO₄, sucrose, CaCl₂, NaHCO₃, acetone, nitrogen, acetic acid, KOH, methanol, HCL, distilled water, ether, hexane, KCl, Na₂SO₄, carbitol, diazold, and all glassware required were obtained from Dr. Alice Bennett's research facilities.

Animals

The mice used for this project were obtained by Dr. Bennett from the late Dr. Gordon L. Rosene, Department of Physiology, Ball State University. The mice originated during the summer of 1921 when Strong obtained two albino mice while working at the Carnegie Institution of Washington, Spring Harbor, Long Island. These mice were mated and the stock was continued by brother-to-sister matings (19).

Until the summer of 1927, all the mice were used for transplantation studies on tumors. But, in 1927 several different albino mice developed spontaneous carcinoma and it was decided that transplantation studies should be discontinued so that primary tumors could be concentrated upon (19).

The mice used for this project are called strain A/St mice

which refers to Strong A mice. The mice were housed in plastic cages in a controlled environment (21-24 c; 12hr light:dark cycle). They were fed five diets: SAF (11.4% linoleic acid, .4% stearic acid), SA-4 (3.0% linoleic acid, 10.3% stearic acid), SA-1 (0.8% linoleic acid, 13.2% stearic acid), CO (3.0% linoleic acid, 0.1% stearic acid) and ST (1.5% linoleic acid, 0.3% stearic acid).

Preparation of Diets

The special diets fed the mice were prepared in such a way that they contained the same percentage of protein per gram of food. All other factors except the percentage of fat remained constant. The caloric difference in the amount of fat in each of the diets was compensated for by the addition of either sucrose or calorie free cellulose. The safflower oil and stearic acid diet were prepared from either a fat free diet purchased from U.S. Biochemicals or from a fat free base prepared from a mixture of basic ingredients and purified fat. The corn oil diet was prepared from a mixture of basic ingredients and corn oil.

1) Corn Oil Base:

Casein-----	585g
Cellulfil-----	135g
Sucrose-----	2273g
Salt Mix XX-----	111g

*mixed in ball mill and then added:

Vitamin Mix-----	68g
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Corn Oil Diet:

Corn Oil Base-----1000g
Corn Oil-----58ml

2) SA-1 Base:

Casein-----820g
Cellufil-----640g
Sucrose-----2273g
Salt Mix XX-----156g
Stearic Acid-----631g

*mixed in ball mill and then added:

Vitamin Mix-----86g

SA-1 Diet:

SA-1 Base-----1000g
Safflower Oil-----11ml

3) SA-4 Base:

This was made by adding Fat-Free Diet to the SA-1 base.
SA-1 Base-----4606g
Fat-Free Diet-----1244g

SA-4 Diet:

SA-4 Base-----1000g
Safflower Oil-----46ml

4) Safflower Oil Diet:

Fat-Free Mix-----500g
Safflower Oil-----97ml

5) Stock Diet:

This diet consisted of Purina Rodent Chow from Ralston
Purina Company, ST. Louis, MO..

Tumor Selection

Two types of tumors were removed from the mice for this project. Tumors were removed when they reached the 1 cm mark and some were also allowed to grow and were removed at a much larger size. This variation in tumor removal times allowed us to measure the fatty acid compositions of the membranes of large and small tumors. This made it possible to measure the difference in membranes at different stages of tumor growth.

Isolation of Membranes

The following procedure is a slight modification of Ray's (20). Once the tumors were obtained they were stored in .25% acetic acid and frozen until ready for use. When ready for isolation the tumor was thawed out and cut into slices of 2-3mm. The pieces were then suspended in isolation medium (.1M KHPO_4 , pH 7.4, 0.25M sucrose) and homogenized with a glass homogenizer. The connective tissue was removed by filtering the masserated sample through glass wool.

The centrifugation process began at this point by first centrifuging the homogenate at 750xg for 10 minutes. The supernate was saved and the pellet was resuspended with isolation medium and centrifuged at 750xg for 10 minutes. The pellet was then discarded and the two supernates were mixed and centrifuged at 4000xg for 10 minutes. The supernate was discarded and the pellet was resuspended in 5-10ml calcium fortified buffer (2mM CaCl_2 , 1mM NaHCO_3 , pH 7.5). The calcium plays a key role in the

maintenance of structural and functional integrity of the membrane (21). The resuspended pellet was centrifuged at 1500xg for 10 minutes. This centrifugation process was repeated two times and each time the supernate was discarded. The pellet could now be stored in 1.2ml of buffer or continued to the next step.

Ultracentrifugation was the next step in plasma membrane isolation. One half milliliter of the previously stored pellet was removed and placed into an ultracentrifuge tube. one milliliter of 1.34 g/ml sucrose was added and the solution was mixed using a vortex mixer. 0.9ml of 1.20 g/ml sucrose was then added followed by 0.9ml of 1.18 g/ml sucrose and finally, 0.9ml of 1.16 g/ml sucrose solution. The sample(s) was then placed in a swinging bucket rotor and centrifuged in a Beckman model L-50 ultracentrifuge at 38,000 rpm for 90 minutes.

After ultracentrifugation, the purified plasma membranes were located at the interface of the 1.18 g/ml and the 1.16 g/ml density layers. With a pasteur pipette, the membranes were gently removed and placed into a glass centrifuge tube. Equal amounts of buffer were added to each tube and the tubes were then vortexed. The solutions were then centrifuged at 5000xg for 10 minutes whereupon, the supernate was discarded and the pellet was resuspended in 2ml fortified buffer. The solution was again centrifuged but at 1500xg for 10 minutes. When the centrifugation was complete the supernate was discarded and the pellet that remained was the purified plasma membrane. The purified membrane was then resuspended in 0.25% acetic acid and then placed in a plastic, conical storage container. The solution was then covered with nitrogen gas and placed in the freezer.

Saponification

Plasma membrane fractions were placed in 15ml round bottom test tubes. Two milliliters of 15% KOH in 85% methanol was then added to each tube. The methanol was used to dissolve lipids. A marble was placed on every tube to prevent evaporation and the tubes were placed in a hot water bath at 85 C for 1 1/2 hours. The tubes were then removed from the hot water bath and HCl was added to make the solution acid to litmus. A white precipitate (KCl) was usually noted and small amounts of water were used to dissolve it.

The saponification procedure allowed us to cleave fatty acids by a base catalyzed reaction. It also turned the fatty acids into a soap with the carboxyl group being soluble in water. The acidification process allowed us to convert the soap back into a fatty acid in preparation for extraction.

Fatty Acid Extraction

Hexane was added to each tube to dissolve the fatty acids and the solution was vortexed whereupon, the bottom aqueous layer was extracted using a pasteur pipette. An equal volume of saturated KCl was then added to each tube and this solution was mixed and the bottom aqueous layer was removed. Also, this process was performed twice to assure that only the fatty acids were being

extracted. A spatula tip full of Na_2SO_4 was added to each tube and the tubes were mixed to remove any excess water. The contents were transferred to another tube where the solution was evaporated with nitrogen.

Methylation

Fatty acids were methylated by using a technique developed by Schlenk and Gellerman (21). The apparatus for this procedure consisted of two tubes with side arms. The arms were connected through rubber tubing to pasteur pipettes which were inserted through rubber stoppers to within 1 cm of the bottom of the tube. The first tube was also connected to a nitrogen source. The rubber tubing and pasteur pipette of the last tube were used to insert into the sample to be methylated.

The first tube was filled with approximately 6ml of ether. The second tube contained 2ml carbitol and a mixture of 2ml Diazold and 2ml ether. The third tube consisted of the sample mixed with 5ml of ether:MeOH. The nitrogen flow rate was adjusted so that the stream would just make the ether bubble. 1-2ml of KOH was then added to the second tube to begin the base catalyzed reaction. Diazomethane was produced which changed our fatty acids into fatty acid methyl esters. The process was complete when there was an excess of diazomethane in the sample and the solution turned yellow. This procedure allowed us to make the fatty acids less polar which allowed them to move more quickly through the column of the Gas Chromatograph.

Gas-Liquid Chromatography

The methyl esters of fatty acids extracted were identified using the Gas-Liquid Chromatograph (Varian 3600). The operating conditions were inlet temperature of 250 C, nitrogen flow rate of 166.5 and column temperature of 195 C. Methyl esters of fatty acids were identified by comparing retention times with standard values Chromatographs were also produced by a varian 4290 integrator. The integrator also recorded the retention time and area of each peak. The final identification of the fatty acids was done by using a computer program, developed by Larry Bennett, which was based on peak retention times of fatty acids.

RESULTS AND CONCLUSIONS

The fatty acid composition of plasma membranes of 24 tumors excised from animals on various diets were determined. Tumors were excised at two different stages. Some tumors were removed when they reached 1 cm in diameter and some were allowed to grow and were removed at a much larger stage. The following tumors were removed:

1. SA-1: 4 small, 3 large
2. CO: 5 small, 2 large
3. SA-4: 2 small, 1 large
4. SAF: 3 small, 2 large
5. ST: 2 small

Analysis of fatty acids of the various membrane fractions are listed in the appendix. Specific values for linoleic acid and stearic acid were calculated and compared. High fat diets such as SA-1, SA-4 and SAF varied in their stearic acid content. SA-4 contained the highest amount of 18:0 with 24.1% in the small tumors and 16.9% in the large tumors as compared to 8.1% in the STOCK tumors. SAF and SA-1 membrane fractions were similar to each other in 18:0 concentrations. SAF contained an average of 8.3% for small tumors and 12.6% for large tumors. SA-1 contained an average of 9.5% for small tumors and 10.9% for large tumors. Low fat diets such as the STOCK and CO diets did not differ very much from the SAF and SA-1 diet.

The membrane fractions from the high fat and low fat diets

were extremely low in linoleic acid (18:2). The most linoleic acid detected in any of the samples was 3.5% which was obtained from the SAF large tumors. This value was only .8% higher than the 18:2 percentage in the STOCK tumors. On the other hand, The SAF small tumors did not contain any 18:2. The rest of the diets ranged from 0.1% to 1.9% which is below the 2.7% for STOCK tumors.

When comparing the effects of the various diets on the fatty acid composition of the plasma membrane it can be shown that the SA-4 diet produces a decrease in the amount of 18:2 and a drastic increase in the amount of 18:0 and 22:2 in the membrane. SAF large tumors also showed an increase in 18:0. The CO diet produced a tremendous decrease in the amount of 18:2 and all the other tumors from the various diets, except SAF large tumors, also showed a decrease in 18:2 concentration.

These results indicate that high fat diets increase the utilization of stearic acid in the development of plasma membranes. High fat diets may also increase tumorigenesis by decreasing the amount of polyunsaturated fats used for membrane development and therefore, decreasing the fluidity of the membrane. Low fat diets showed a small increase in stearic acid utilization and a decrease in linoleic acid utilization. Since the membranes from the mice fed a low fat diet contained less stearic acid, they should be more fluid than the membranes from the mice fed high fat diets thus less tumorigenic. The large decrease in linoleic acid may be attributed to early activities in tumor development such as prostaglandin development.

FATTY ACID CONTENT(%) FOR LARGE TUMORS

*****					*****				
* high fat					* low fat				
* SAF SA-4 SA-1 CO					* CO				
* 16:0	15.5 \pm 3.60	21.4	16.4 \pm 4.63		14.8 \pm 0.2				
* 16:1	5.80 \pm 2.25	4.6	5.00 \pm .967		5.10 \pm 0.1				
* 16:2	0.95 \pm 0.95	0	1.80 \pm 1.29		0				
* 18:0	12.6 \pm 3.35	16.9	10.9 \pm 3.87		8.70 \pm 0.8				
* 18:1	16.2 \pm 1.25	10.6	7.80 \pm 2.61		5.20 \pm 1.30				
* 18:2	3.50 \pm 1.30	1.9	1.80 \pm .189		0.60 \pm 0.55				
* 18:3	0	0	0		0				
* 18:7	0	0	0		0				
* 22:1	0.80 \pm 0.80	5.0	0		0				
* 22:2	1.90 \pm 0.30	6.8	6.70 \pm 4.12		3.80 \pm 0.95				
* 22:3	0	0	0		0				
* 22:4	0.30 \pm 0.30	0	0		0				
* 22:6 ¹	0	0	0		2.70 \pm 2.70				
*****					*****				

¹The first number equals the number of carbon molecules and the second number is the number of double bonds.

FATTY ACID CONTENT(%) FOR SMALL TUMORS

*****						*
*	high fat			low fat		*
*						*
*	SAF	SA-4	SA-1	CO	STOCK	*
*16:0	13.8 <u>±</u> 4.19	24.3 <u>±</u> 0.80	17.1 <u>±</u> 5.24	14.6 <u>±</u> 4.49	14.7 <u>±</u> 3.75	*
*						*
*16:1	2.40 <u>±</u> 1.85	1.90 <u>±</u> 1.85	2.80 <u>±</u> 2.84	4.90 <u>±</u> 2.32	4.10 <u>±</u> 1.00	*
*						*
*16:2	1.10 <u>±</u> 1.60	0	1.70 <u>±</u> 2.81	0	1.10 <u>±</u> 1.10	*
*						*
*18:0	8.30 <u>±</u> .736	24.1 <u>±</u> 9.05	9.50 <u>±</u> 2.22	9.80 <u>±</u> 3.43	8.10 <u>±</u> 1.45	*
*						*
*18:1	3.90 <u>±</u> .822	5.20 <u>±</u> 2.35	6.50 <u>±</u> 1.91	5.40 <u>±</u> 2.32	6.50 <u>±</u> 0.70	*
*						*
*18:2	0	0.60 <u>±</u> 0.60	1.80 <u>±</u> 2.77	0.10 <u>±</u> 0.20	2.70 <u>±</u> 0.95	*
*						*
*18:3	0	0	.480 <u>±</u> .822	0	0	*
*						*
*18:7	0	0	.730 <u>±</u> 1.26	0	0	*
*						*
22:1	.230 <u>±</u> .329	0.70 <u>±</u> 0.70	0	.660 <u>±</u> 1.32	0.10 <u>±</u> 0.10	*
*						*
*22:2	2.40 <u>±</u> 1.39	9.30 <u>±</u> 6.55	2.70 <u>±</u> 1.38	2.20 <u>±</u> 1.12	3.90 <u>±</u> .450	*
*						*
*22:3	0	0	0	0	0.05 <u>±</u> 0.05	*
*						*
*22:4	0	0	0	0	0	*
*						*
*22:6 ¹	0	0	0	0	0	*

¹The first number equals the number of carbon molecules and the second number is the number of double bonds.

SUMMARY

The plasma membrane fractions that were analyzed showed an interesting variance in the amount of 18:0 and 18:2. SA-4 showed a quite large percentage of 18:0 and a very low percentage of 18:2. All the tumors showed an increase in the amount of 18:0 as compared to the STOCK diet tumors. All the tumors also showed a decrease in the amount of 18:2 except the SAF large tumors which had a very small increase. These results indicate that the linoleic acid must be being used elsewhere with stearic acid being used to develop the plasma membranes.

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APPENDIX

FATTY ACID CONTENT(%) FOR SAF

* SAF small SAF large *							
	q23	q24	q44	AVERAGE	q48	q33	AVG
*16:0	13.6	9.5	18.4	13.8 \pm 4.19	11.9	19.1	15.5 \pm 3.60
*16:1	4.5	0	2.8	2.4 \pm 1.85	3.5	8.0	5.8 \pm 2.25
*16:2	0	3.4	0	1.1 \pm 1.60	0	1.9	.95 \pm .95
*18:0	7.4	8.2	9.2	8.3 \pm .736	9.2	15.9	12.6 \pm 3.35
*18:1	4.1	2.9	4.9	3.9 \pm .822	4.9	7.4	6.2 \pm 1.25
*18:2	0	0	0	0	2.2	4.8	3.5 \pm 1.30
*18:3	0	0	0	0	0	0	0
*18:7	0	0	0	0	0	0	0
*22:1	0	0.7	0	.23 \pm .329	0	1.6	.8 \pm .80
*22:2	3.4	0.4	3.3	2.4 \pm 1.39	1.6	2.2	1.9 \pm .30
*22:3	0	0	0	0	0	0	0
*22:4	0	0	0	0	0.6	0	0.3 \pm 0.3
*22:6 ¹	0	0	0	0	0	0	0

¹The first number equals the number of carbon molecules and the second number is the number of double bonds.

FATTY ACID CONTENT(%) FOR SA-4

*****					*****				
* SA-4 small					* SA-4 large				
* q27 q29 AVERAGE q35					* q35				
*16:0	25.1	23.5	24.3±0.8		21.4				
*16:1	0	3.7	1.9±1.85		4.6				
*16:2	0	0	0		0				
*18:0	33.1	15.0	24.1±9.05		16.9				
*18:1	2.8	7.5	5.2±2.35		10.6				
*18:2	0	1.2	0.6±0.6		1.9				
*18:3	0	0	0		0				
*18:7	0	0	0		0				
*22:1	0	1.4	0.7±0.7		5.0				
*22:2	2.7	15.8	9.3±6.55		6.8				
*22:3	0	0	0		0				
*22:4	0	0	0		0				
*22:6 ¹	0	0	0		0				

¹The first number equals the number of carbon molecules and the second number is the number of double bonds.

FATTY ACID CONTENT(%) FOR SA-1

*	SA-1 small				SA-1 large				*
*	q7	q8	q30	q43	AVERAGE	q11	q12	q31	AVG
*16:0	9.2	23.9	18.2	17.0	17.1 \pm 5.24	13.4	12.8	22.9	16.4 \pm 4.63
*16:1	0	0	6.1	5.2	2.8 \pm 2.84	4.3	4.4	6.4	5.0 \pm .967
*16:2	6.5	0	0	0	1.7 \pm 2.81	2.8	2.7	0	1.8 \pm 1.29
*18:0	6.5	11.1	12.1	8.3	9.5 \pm 2.22	9.0	7.4	16.3	10.9 \pm 3.87
*18:1	4.8	9.2	7.4	4.6	6.5 \pm 1.91	5.1	7.3	11.4	7.8 \pm 2.61
*18:2	0	6.6	0.7	0	1.8 \pm 2.77	2.1	1.7	1.7	1.8 \pm .189
*18:3	0	1.9	0	0	.48 \pm .822	0	0	0	0
*18:7	0	2.9	0	0	.73 \pm 1.26	0	0	0	0
*22:1	0	0	0	0	0	0	0	0	0
*22:2	0.3	3.8	3.3	3.2	2.7 \pm 1.38	4.3	3.3	12.5	6.7 \pm 4.12
*22:3	0	0	0	0	0	0	0	0	0
*22:4	0	0	0	0	0	0	0	0	0
*22:6 ¹	0	0	0	0	0	0	0	0	0

¹The first number equals the number of carbon molecules and the second number is the number of double bonds.

FATTY ACID CONTENT(%) FOR CO

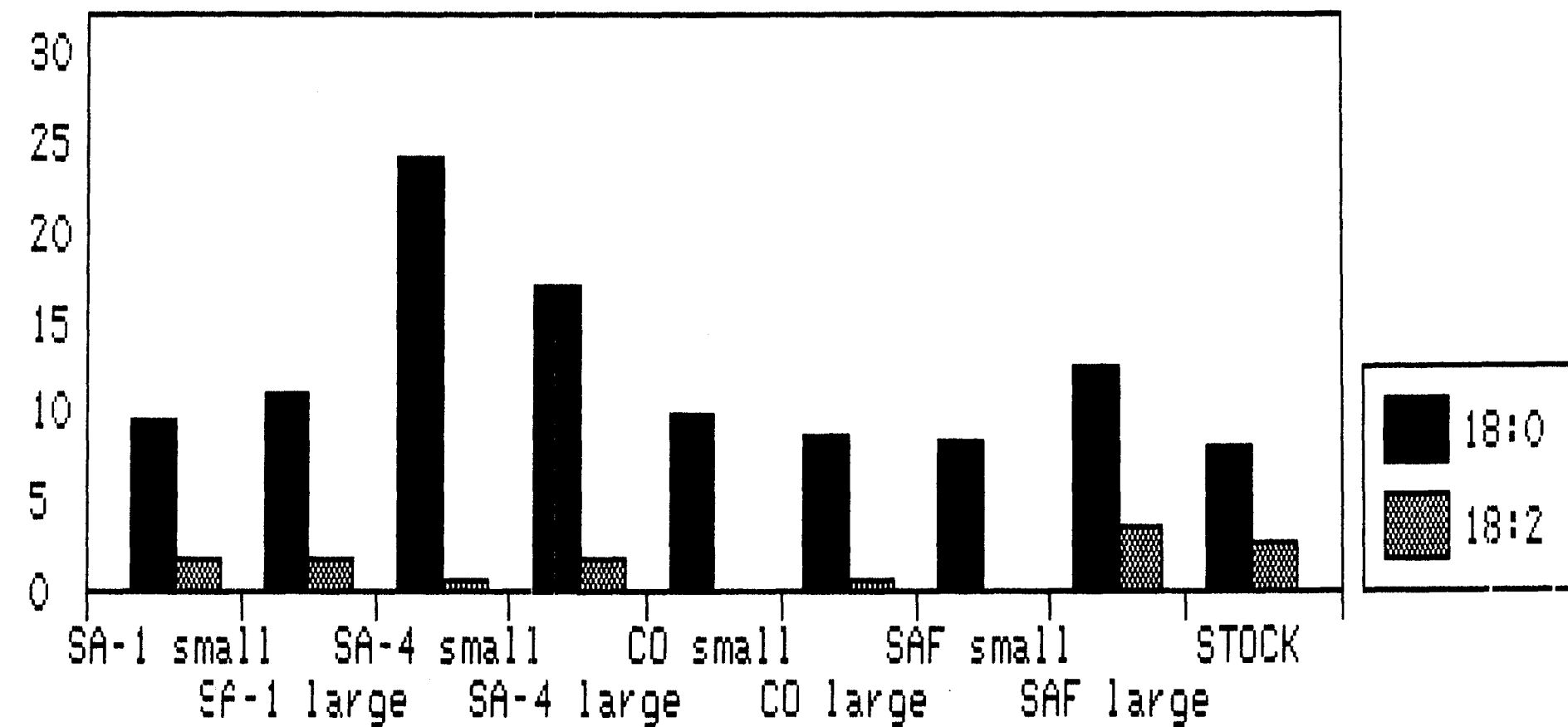
* * * * *									
	CO small						CO large		
	q22	q25	q26	q46	q47	AVERAGE	q34	q37	AVG
*16:0	9.4	16.7	22.2	13.0	11.6	14.6 \pm 4.49	15.0	14.6	14.8 \pm 0.2
*16:1	4.3	9.5	3.4	3.8	3.5	4.9 \pm 2.32	5.0	5.2	5.1 \pm 0.1
*16:2	0	0	0	0	0	0	0	0	0
*18:0	5.4	13.1	14.5	8.7	7.5	9.8 \pm 3.43	9.5	7.9	8.7 \pm 0.8
*18:1	6.5	4.4	1.4	8.1	6.6	5.4 \pm 2.32	3.9	6.5	5.2 \pm 1.3
*18:2	0	0	0	0	0.5	0.1 \pm 0.2	0	1.1	0.6 \pm .55
*18:3	0	0	0	0	0	0	0	0	0
*18:7	0	0	0	0	0	0	0	0	0
*22:1	0	0	3.3	0	0	.66 \pm 1.32	0	0	0
*22:2	4.1	1.2	0.9	2.3	2.3	2.2 \pm 1.12	4.7	2.8	3.8 \pm .95
*22:3	0	0	0	0	0	0	0	0	0
*22:4	0	0	0	0	0	0	0	0	0
*22:6 ¹	0	0	0	0	0	0	5.3	0	2.7 \pm 2.7

¹The first number equals the number of carbon molecules and the second number is the number of double bonds.

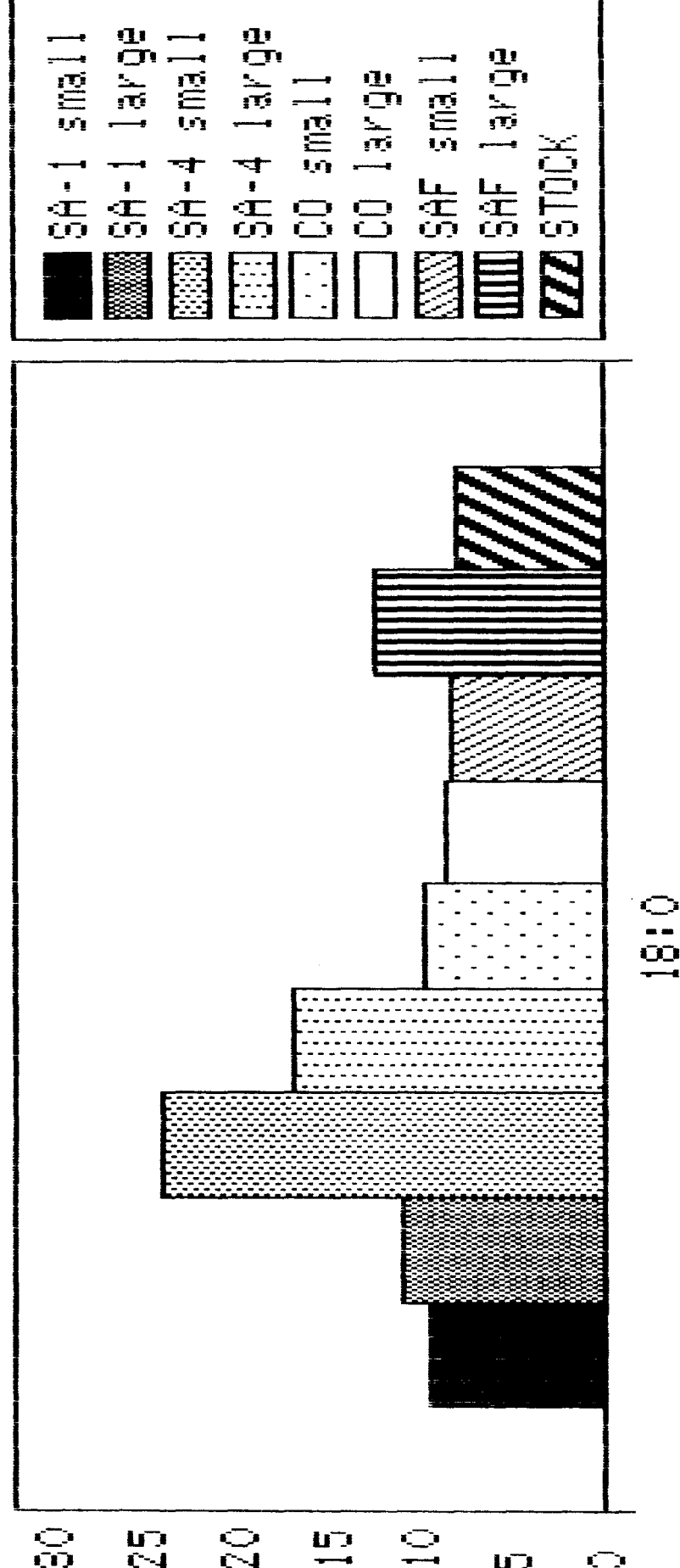
FATTY ACID CONTENT(%) STOCK

*****				*****			
*				*			
*				*			
				STOCK			
*				*			
*				*			
	q5	q6	AVERAGE				
*16:0	10.9	18.4	14.7 <u>±</u> 3.75				
*				*			
*16:1	3.1	5.1	4.1 <u>±</u> 1.00				
*				*			
*16:2	2.1	0	1.1 <u>±</u> 1.10				
*				*			
*18:0	6.6	9.5	8.1 <u>±</u> 1.45				
*				*			
*18:1	5.8	7.2	6.5 <u>±</u> 0.70				
*				*			
*18:2	3.6	1.7	2.7 <u>±</u> 0.95				
*				*			
*18:3	0	0	0				
*				*			
*18:7	0	0	0				
*				*			
*22:1	0.2	0	0.1 <u>±</u> 0.10				
*				*			
*22:2	4.4	3.5	3.9 <u>±</u> .450				
*				*			
*22:3	0.1	0	0.05 <u>±</u> 0.05				
*				*			
*22:4	0	0	0				
*				*			
*22:6 ¹	0	0	0				
*				*			

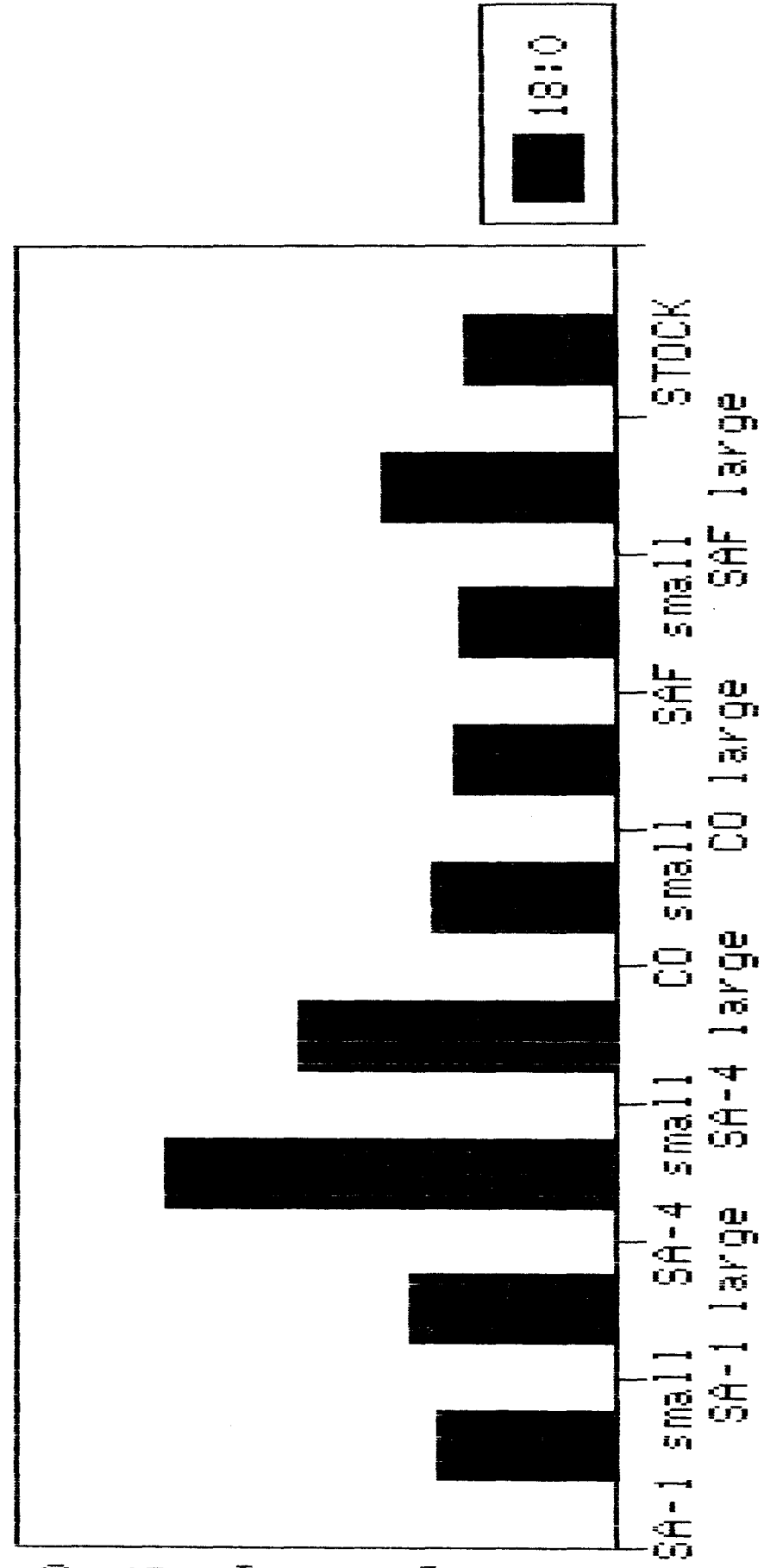
Amount of 18:0 and 18:2



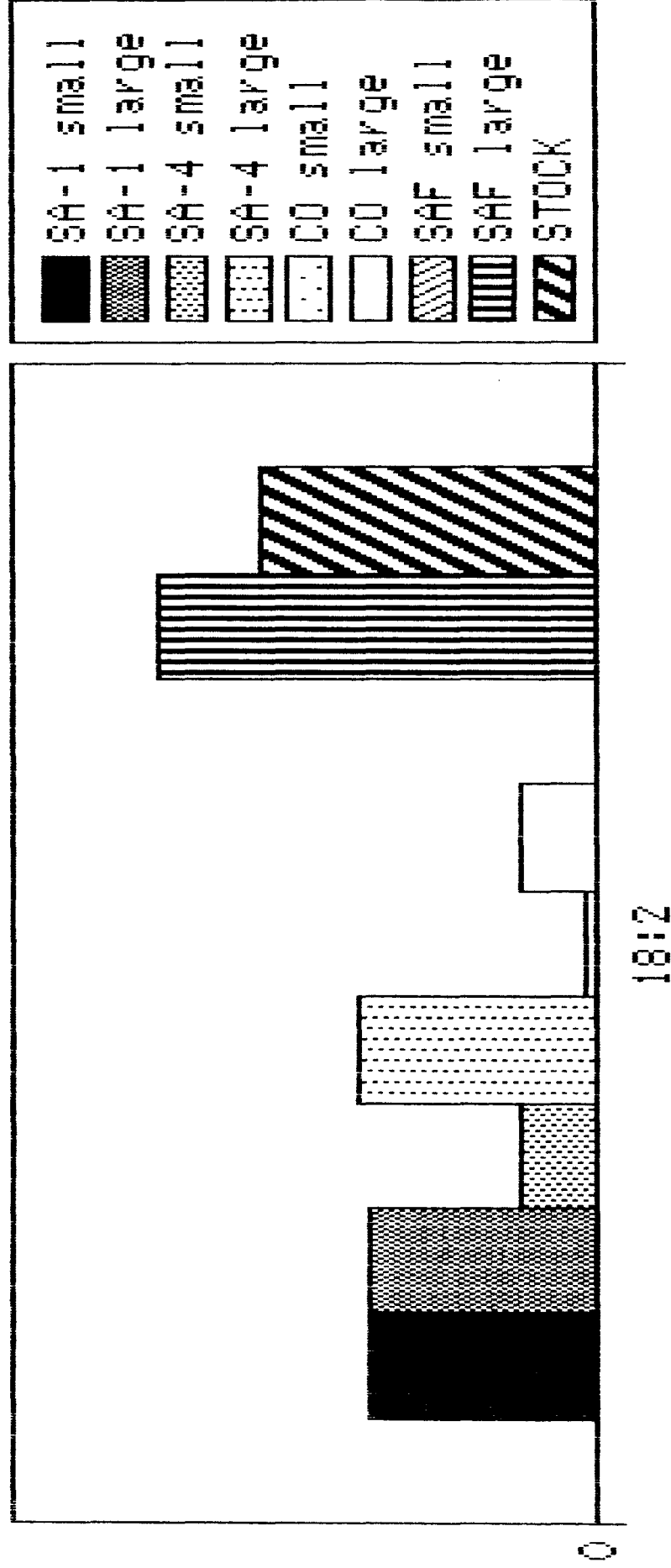
Percent Stearic Acid



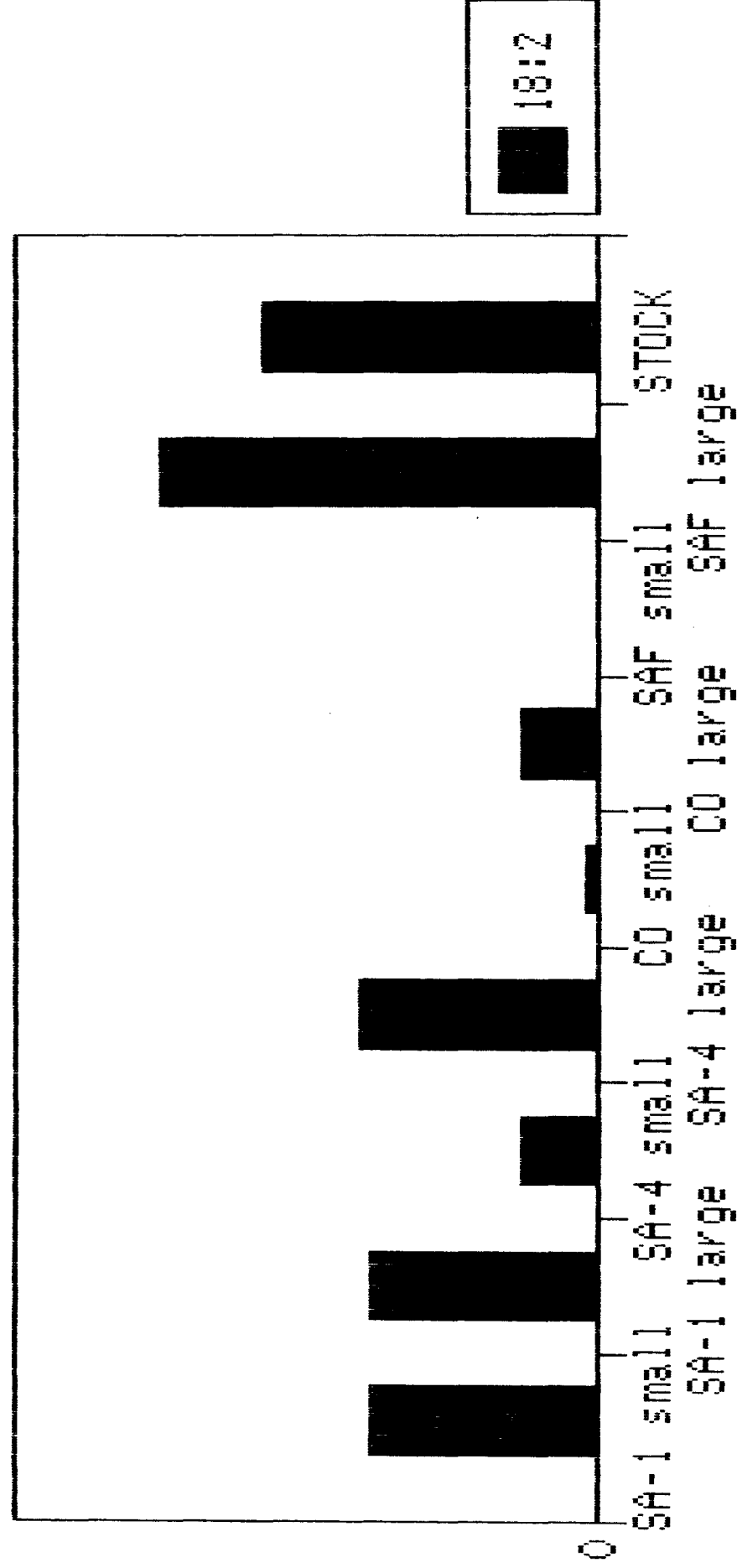
Percent Stearic Acid



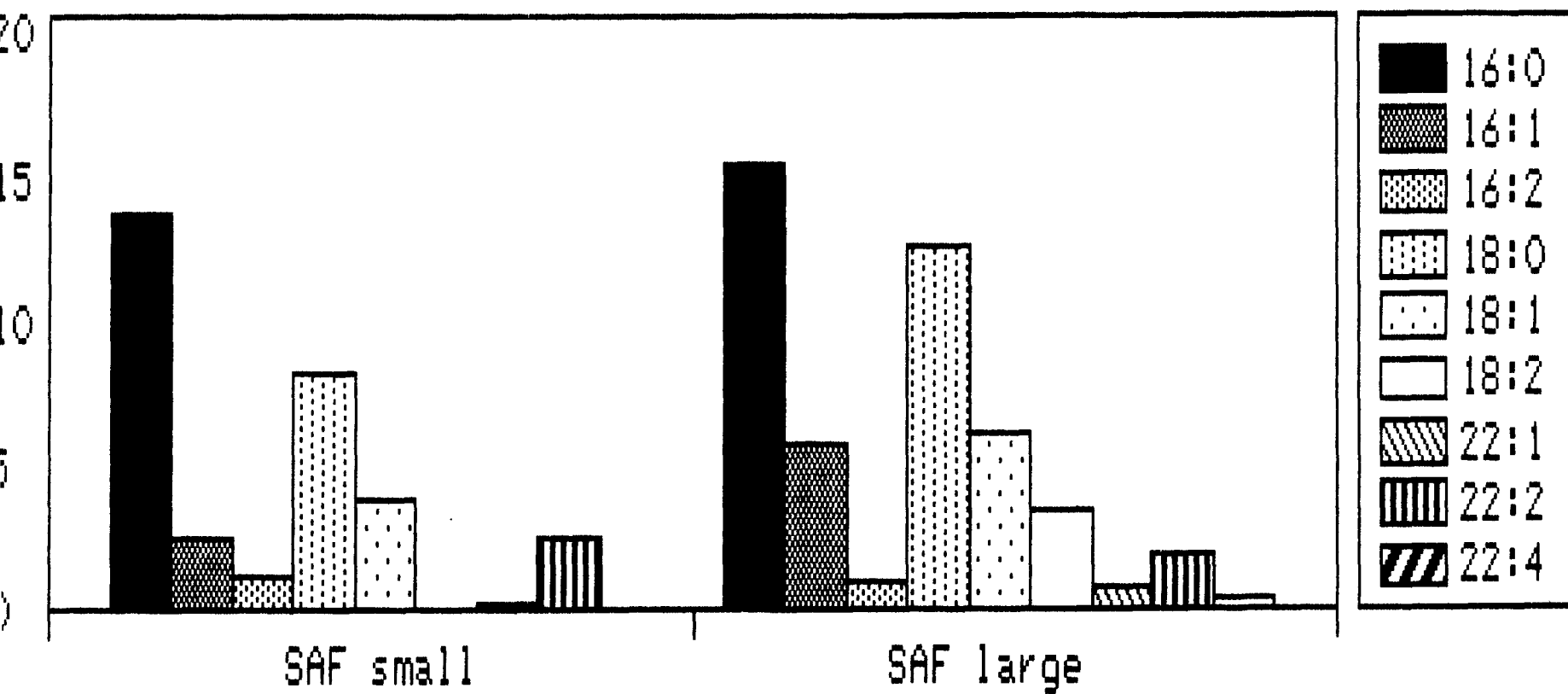
Percent Linoleic Acid



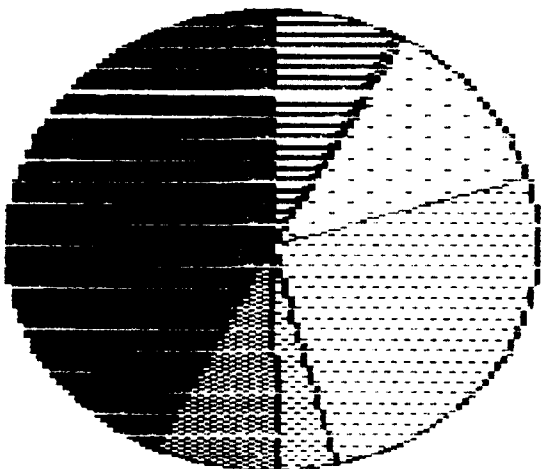
Percent Linoleic Acid



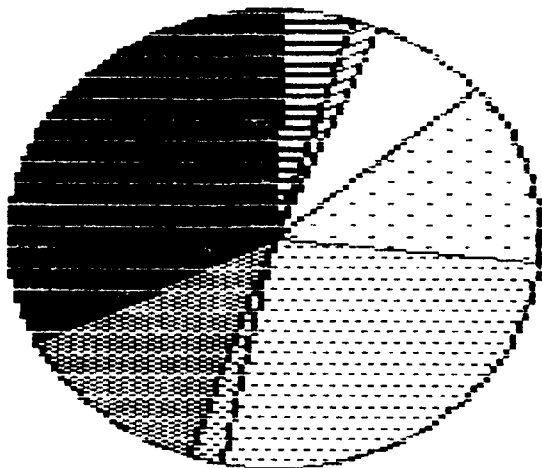
Percent Fatty Acid



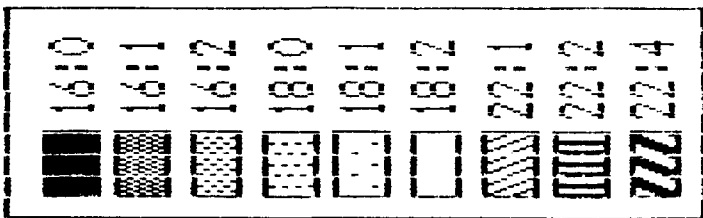
Percent Fatty Acid



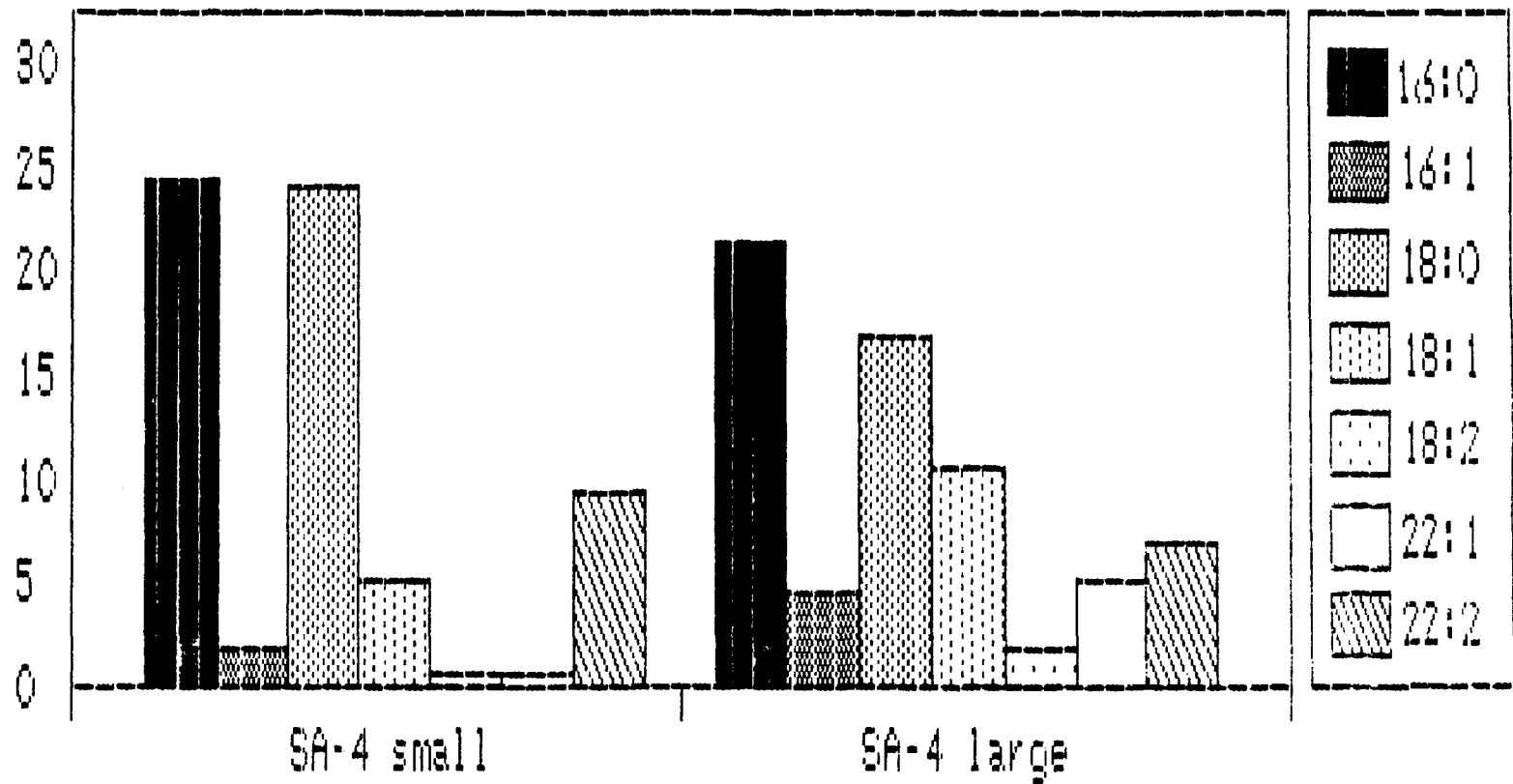
SAF small



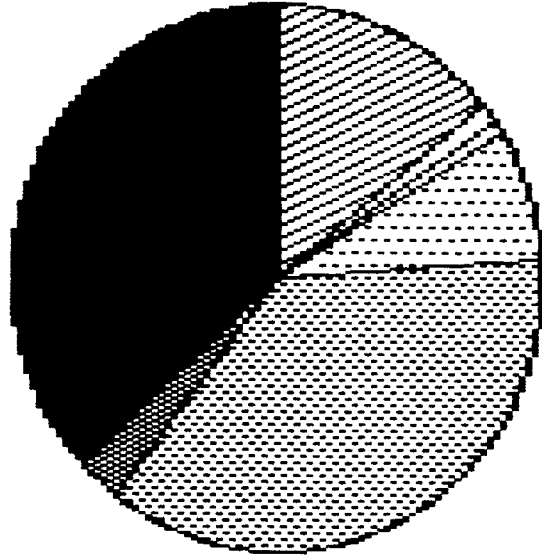
SAF large



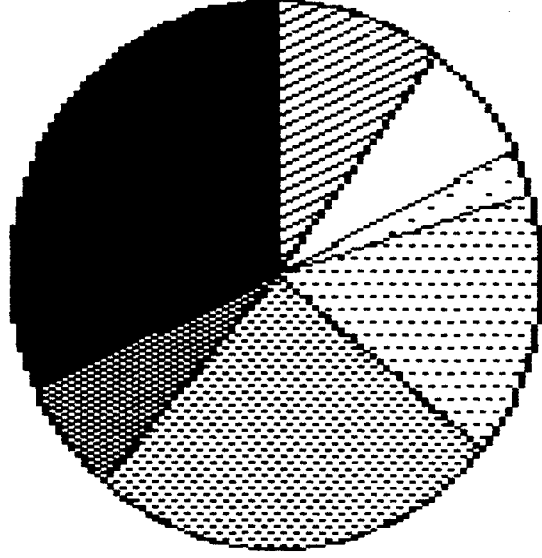
Percent Fatty Acid



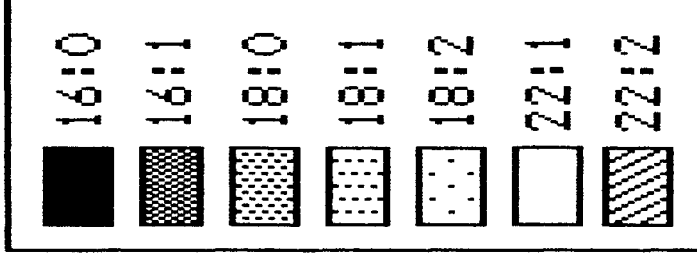
Percent Fatty Acid



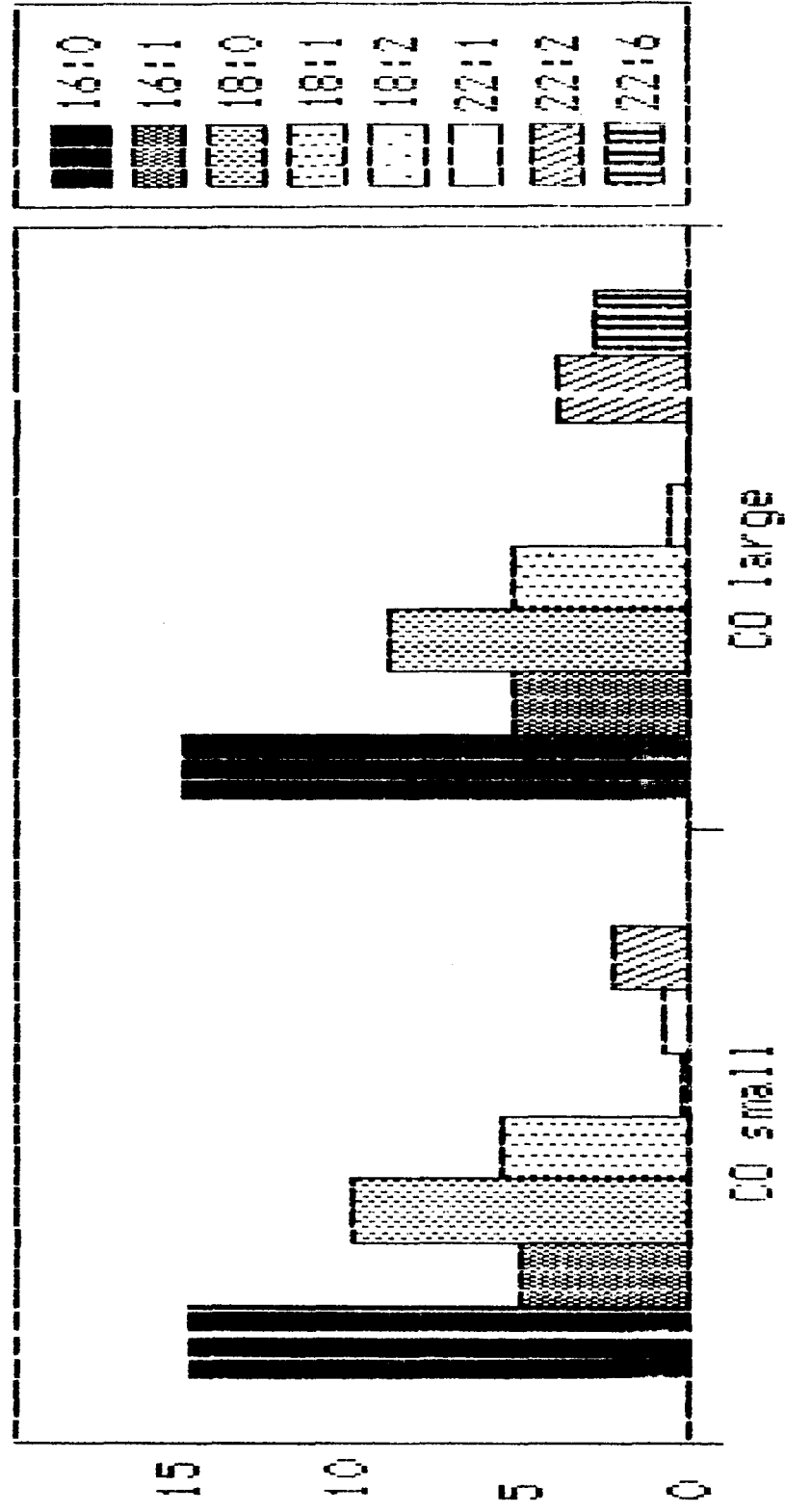
SA-4 small



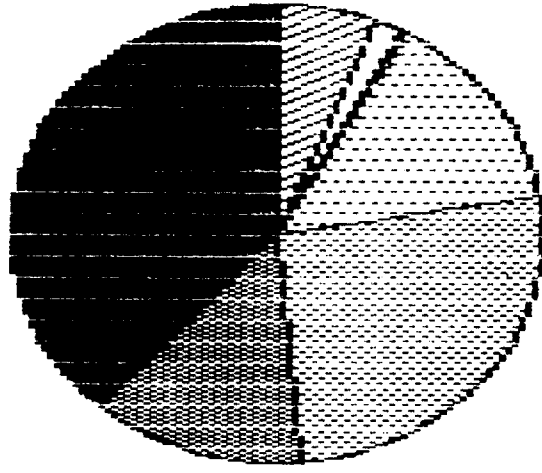
SA-4 large



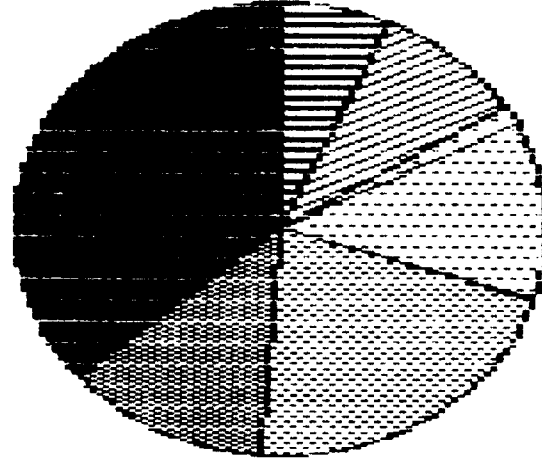
Percent Fatty Acid



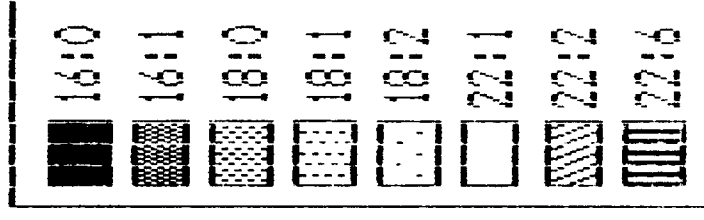
Percent Fatty Acid



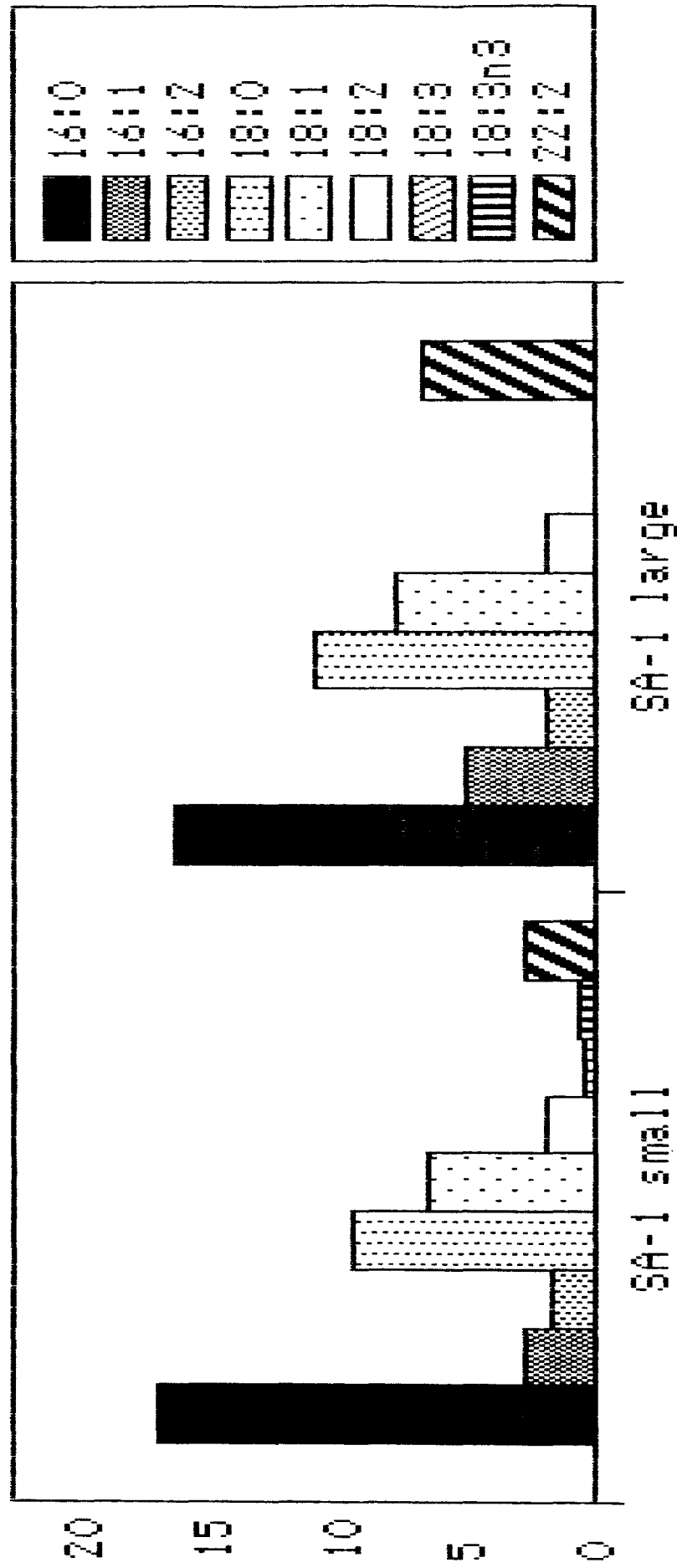
small



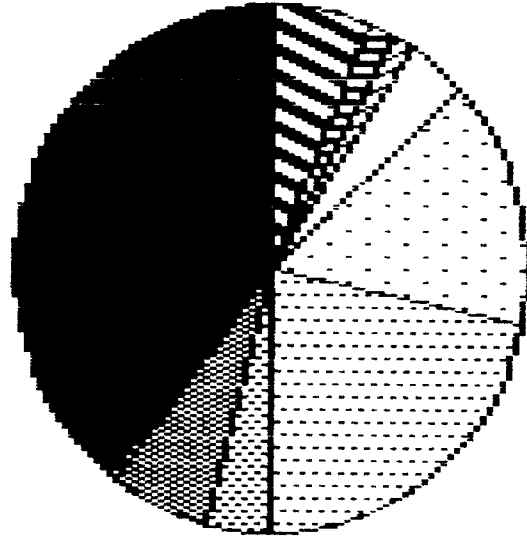
large



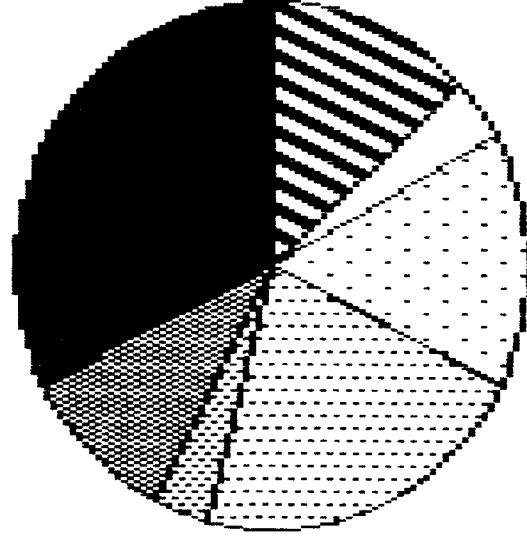
Percent Fatty Acid



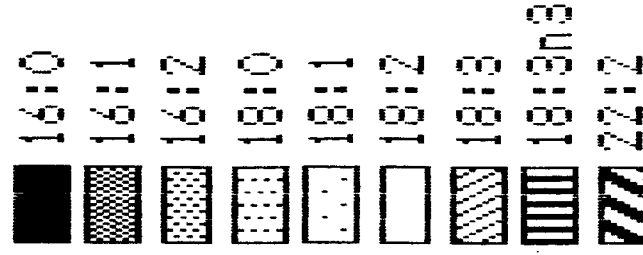
Percent Fatty Acid



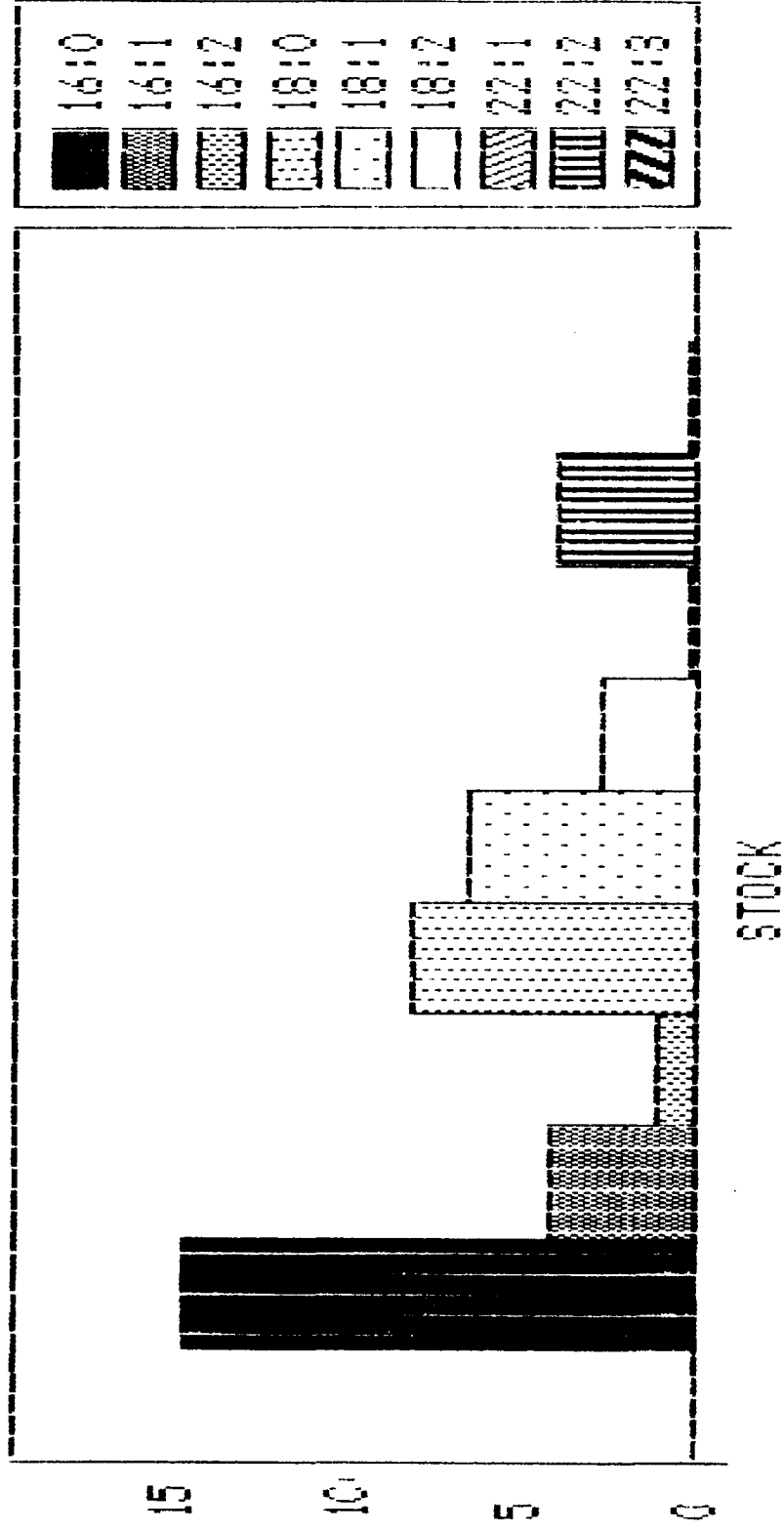
SA-1 small



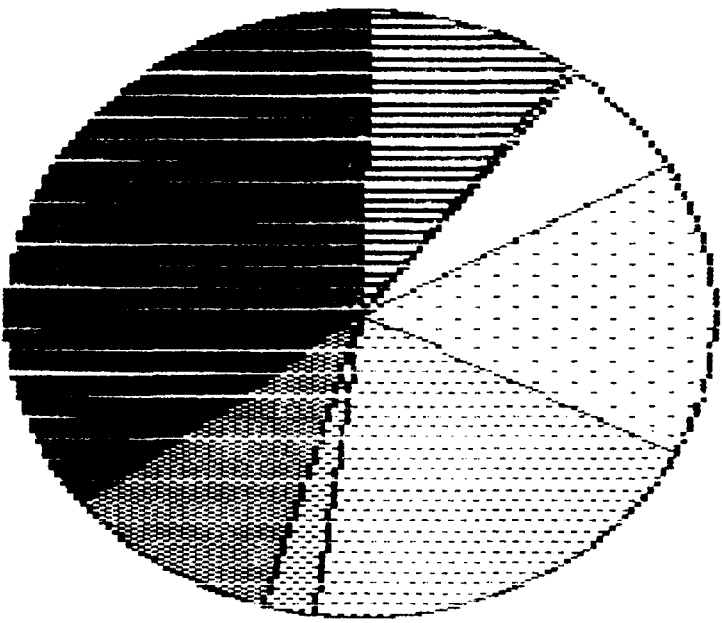
SA-1 large



Percent Fatty Acid



Percent Fatty Acid



STOCK

	16:0
	16:1
	16:2
	18:0
	18:1
	18:2
	22:1
	22:2
	22:3